

Incidence of Aflatoxin in California Almonds

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In a survey of California almonds, aflatoxin was found in 14% of 74 samples of unsorted, in-shell almonds as received by the processor in 1972, but it occurred at very low levels (below 20 parts per billion [ppb]) in 90% of the contaminated samples. The overall proportion of individual nuts contaminated was especially low and is estimated with 95% probability to have been in the range of 1 nut/55,300 nuts to 1 nut/14,700 nuts. Aflatoxin contamination is not restricted to any particular section of the almond-growing region of California. Commercial sorting procedures are effective in removing most aflatoxin-contaminated nutmeats, since none of 26 samples of processed, whole nutmeats contained aflatoxin. In contrast, 13 of 27 samples of diced almonds were contaminated, but nine of these 13 samples contained less than 20 ppb. Only one of 25 samples of sliced nutmeats contained aflatoxin (4 ppb). Thus, aflatoxin incidence in almonds varies greatly with the category of finished product. The apparent high incidence in diced nutmeats is probably due mostly to the more uniform distribution of aflatoxin occurring in this product (because of its small particle size) than that occurring in the other products. Sample size requirements for monitoring aflatoxin in almonds are discussed.

Aflatoxins may occur in food products if certain molds, namely *Aspergillus flavus* or *A. parasiticus*, develop on them under appropriate conditions. These molds, like other storage fungi, seem especially likely to be a problem when seeds or nuts are at an intermediate moisture level, i.e., below that required by field fungi and above that inhibitory to fungal growth (4). The minimum moisture level for aflatoxin production at 30 C by *A. flavus* is equal to the moisture content of a product in equilibrium with 83% relative humidity or higher, depending on the nature of the substrate and the duration of storage (6). For starchy cereal seeds such as maize and wheat, the limiting moisture level for growth of *A. flavus* is about 18.5% (4), whereas in oily seeds such as peanuts it is 8 (4) or 9% (7). For almonds (*Prunus amygdalus*), the limiting moisture content for growth of *A. flavus* is likely to be similar to that found for peanuts, due to the similarity in composition of these nuts.

Infection of tree nuts with aflatoxigenic molds probably occurs most often in the field before and/or during harvest while the kernels are still moist. Tree nuts are generally exposed to field dirt and to possible physical damage by modern

methods of mechanical harvesting, which involve knocking the nuts to the ground and later collecting them with a machine that brushes them onto a conveyor belt traveling in front of a collecting bin (8). Insect damage before and after harvest might contribute to the invasion and development of molds. The exact conditions responsible for the contamination of tree nuts remain to be established.

Since aflatoxins are highly toxic to most animals and carcinogenic to at least some animal species (12), their presence in various food and feed crops poses a serious threat to the safety of our foods. Foods subject to aflatoxin contamination have been regulated by the U.S. Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act, as amended 1972, Section 402a, which relates to adulterated foods (3). Although no legal tolerance has been established as yet for these toxic compounds in foods, the FDA has set an informal guideline level of 20 ppb of aflatoxin, beyond which they will seize a product (1). The guideline level applies to the total amount of aflatoxins, which by analytical methods used routinely by the FDA includes aflatoxins B₁, B₂, G₁, and G₂ (2).

In 1971, the FDA alerted the almond industry of California that the results of a recent FDA study indicated that almonds, as well as other

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tree nuts, are sometimes contaminated with aflatoxins. Therefore, the present study was undertaken with the support of the California almond industry to determine the incidence of aflatoxin in almonds and to determine the effects of commercial handling and processing practices on aflatoxin incidence. This study includes almonds as they arrive from the grower, almonds at various stages of sorting in the processing plant, and almonds as finished products marketed by the processor. Since studies on aflatoxin in peanuts have shown that only a few nuts out of many thousands need to be contaminated to be a serious problem (5, 9-11), it was assumed that very large samples of almonds would be required for representative sampling and meaningful analysis. The sample size chosen represents a compromise between the estimated appropriate size and a size that is practicable in regards to cost and analysis.

MATERIALS AND METHODS

Samples. Samples from the 1972 almond crop were collected throughout the harvesting and processing season. The following general categories of almonds were sampled: (i) unsorted in-shell nuts, representing incoming almonds as received by the processor; (ii) "in-process nuts," representing nutmeats at various stages of sorting by the processor; and (iii) processed nutmeats, representing various finished products sold for food use. This last category consisted of diced, sliced, and whole nutmeats, including the whole nutmeat samples from the final stage of the in-process or sorting study. Only Nonpareil variety almonds were used for the study of unsorted in-shell nuts and of whole nutmeats, because this variety represents the major portion of the California almond crop and because this variety seems most likely to have mold damage due to its extra soft, thin shell. With sliced and diced nutmeats it was not possible to limit samples to varieties, since they are usually prepared from mixed varieties.

The nut samples were obtained from six almond processors of large and medium size, who together handle most of the California almond crop. The unsorted in-shell nuts and the in-process nutmeats were obtained from all six processors, whereas the sliced and diced nutmeats were collected from only three processors. The geographical origin of each in-shell sample was recorded as it was collected. Altogether 223 samples of in-shell nuts and nutmeats were assayed.

Sample handling and preparation. Samples were collected in plastic bags and stored at 32 to 34 F (0 to 1 C) as soon as received at the laboratory to arrest any aflatoxin formation. Storage at low temperature was probably not critical, because all samples received were dry enough (e.g., below 7% moisture) to be fairly safe from mold and aflatoxin development, at least over short periods of storage. The samples were removed from cold storage 1 to several days before being prepared for assay to allow them to reach room

temperature and thereby avoid condensation on the nuts.

In-shell samples were sorted by hand to remove foreign materials, i.e., loose hulls, loosely attached hulls, rocks, sticks, etc. Removal of the variable amounts of foreign materials made the samples more comparable, was necessary to protect the blades of the equipment used for sample preparation, and avoided interference in the analysis by certain constituents of the hulls.

In all but a few cases, there was sufficient sample available to permit 18.0 lb (ca. 8.2 kg) of in-shell nuts or 15.0 lb (ca. 6.8 kg) of nutmeats to be cut and blended in a Hobart vertical cutter-mixer (VCM) (25-qt [about 22.3 liters] VCM). A fine, homogeneous meal was prepared with the VCM by intermittent cutting (e.g., 15 s at a time) at slow speed for 1 min and then at high speed for another 1 or 1.5 min. Allowing the samples to cool between the periodic cuttings avoided problems of overheating, oiling-out, and compacting of the product. In a few cases, it was necessary to use Celite Hyflo Supercel (Johns Mansville) as a cutting aid to attain a finely cut, free-flowing meal. Using a sharp wave-cut blade of the type available for the Hobart VCM contributed to the preparation of a fine nut meal without aid of the diatomaceous earth.

Analysis. Aflatoxins B₁, B₂, G₁, and G₂ were determined by a procedure very similar to Method I for aflatoxins in peanuts given in the Official Methods of Analysis of the AOAC (2). Method I has been found by the FDA and ourselves to be the most reliable method for tree nuts of the several official aflatoxin methods. The analysis was carried out under gold fluorescent lighting to avoid possible loss of the ultraviolet-sensitive aflatoxins. A weighed portion of the finely cut samples of nutmeats (50.0 g) or in-shell nuts (75.0 g, which is equivalent to about 50.0 g of nutmeats) was mixed with 15 g of Celite Hyflo Supercel, 25 ml of water, and 250 ml of chloroform in a 500-ml, glass-stoppered (g.s.) Erlenmeyer flask. If cutting aid was present in the sample, the sample size was increased proportionately. After shaking the sealed flask and its contents on a Burrell wrist-action shaker for 30 min, the extract was filtered rapidly through a Buchner funnel containing filter paper (Schleicher and Schuell no. 595) coated with 10 g of Celite Hyflo Supercel. A 50-ml sample of the filtrate was chromatographed on a silica gel column, and the eluate was analyzed by thin-layer chromatography according to the AOAC procedure. The aflatoxins were estimated quantitatively against aflatoxin standards obtained from the Southern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La.

Analysis of subsamples. The method of analyzing the 50 g of subsamples used to study aflatoxin distribution differed from the usual method slightly in sample preparation and extraction. Thus, 50 g of the sample (whole nutmeats or diced nutmeats) was used as received. The sample together with 15 g of Celite Hyflo Supercel was ground in a Waring blender (1-qt jar [about 0.946 liters]) with 250 ml of chloroform for 1 min. The mixture was transferred to a 500-ml g.s. flask, 25 ml of distilled water was added, and the

extraction was continued on a wrist-action shaker for 30 min. The extract was filtered and analyzed as described in the method used for the survey.

RESULTS AND DISCUSSION

Unsorted in-shell nuts. The survey of incoming in-shell nuts was expected to give some idea of the incidence and level of aflatoxin in Nonpareil almonds as received at the processing plant. Although the overall concentration of aflatoxin in these nuts appears to have been fairly low, aflatoxin was by no means a rare contaminant. About 14% of the samples (10 of 74) were contaminated with aflatoxin at 1 ppb or more. The amounts of aflatoxin found in the positive samples are shown in Table 1. It should be noted that the concentrations in this table are expressed on a total weight basis (i.e., kernel plus shell). Whereas only one sample of in-shell nuts exceeded the present guideline of 20 ppb (edible portion), four other samples contained 5 ppb or more of aflatoxin.

Since aflatoxin incidence in in-shell almonds might be related to orchard location, the geographical origin of each contaminated sample was examined. The 10 almond samples containing aflatoxin originated in all sections (i.e., northern, central, and southern sections) of the growing area in the Central Valley of California. Although a more extensive study might show some correlation of aflatoxin incidence to climatic conditions or cultural practices within the almond-growing area of California, it is evident that the aflatoxin problem is not restricted to any one district of California.

Estimation of proportion of contaminated nuts. It must be emphasized that, although the proportion of samples found contaminated was

substantial (14%), the proportion of individual nuts contaminated was probably exceedingly small, assuming aflatoxin incidence in almonds is similar to that found in peanuts (5, 10). The proportion of contaminated nuts can be estimated by statistical analysis. The probability of a contaminated sample can be estimated by the Poisson distribution:

$$1 - e^{-kp} \quad (1)$$

where: k = number of nuts in the sample and p = proportion of contaminated nuts. A point estimate of the proportion of contaminated nuts (p) can be obtained by equating this function (1) to the proportion of positive samples (x) actually found in n samples.

$$x/n = 1 - e^{-kp}$$

solving for p :

$$p = [\ln(1 - x/n)] / -k \quad (2)$$

Since (1) defines the probability of one of two possible outcomes, it is appropriate to use the standard formulas for binomial confidence intervals on this function. The resulting upper and lower limits are each solved for p .

The average proportion of contaminated nuts (p) and its 95% confidence intervals (upper and lower limits) can be estimated for the unsorted in-shell nuts, if it is assumed that all samples had 240 in-shell nuts/lb (or per 453.6 g). Thus, it is estimated that the average proportion of nuts contaminated with aflatoxin for the unsorted in-shell nuts is 3.78×10^{-5} and that the lower and upper 95% confidence limits are 1.8×10^{-5} and 6.7×10^{-5} , respectively. The reciprocal of the proportion of contaminated nuts, which gives the number of good nuts per contaminated nut, provides a better visual picture of the small proportion of nuts that are contaminated. Thus, it is estimated that on the average only one nut in 26,500 nuts (about one nut in 110 lb [about 49.9 kg] of in-shell almonds) is contaminated; the 95% confidence interval is one nut per 55,300 nuts to one nut per 14,700 nuts.

In-process nuts. The survey of nutmeats taken at various stages of commercial sorting and grading shows that normal sorting procedures, which are based on physical appearance, reduce the incidence and levels of aflatoxin in almonds. The results show that manual sorting tends to remove the aflatoxin-contaminated kernels from the sorted (select) kernels, resulting in their concentration in the rejected kernels (Table 2). None of the 26 samples of sorted kernels showed any sign of aflatoxin, whereas 10

TABLE 1. Aflatoxin-contaminated samples of unsorted, in-shell almonds

Sample	Aflatoxin (ppb, total wt basis) ^a				
	B ₁	B ₂	G ₁	G ₂	Total
1	42	0	62.5	2.5	107
2	10	2.5	0	0	12.5
3	6.2	3.8	0	0	10
4	5	1.5	0	0	6.5
5	4	1	0	0	5
6	2	0	0	0	2
7	1.5	± ^b	0	0	1.5
8	1	0	0	0	1
9	1	0	0	0	1
10	0.5	±	0	0	1

^a Kernel weight is approximately 65 to 70% of total weight.

^b ±, Trace.

TABLE 2. *Aflatoxin incidence in in-process samples of whole Nonpareil nutmeats*

Sample description	Proportion of positive samples (x/n)	Total aflatoxin concn	
		Individual positive samples (ppb)	Avg for sample category (ppb)
Unsorted kernels	2/16	10, 47	3.5
ESM ^a -rejected kernels	4/12	9, 24, 226, 272	44.2
ESM ^a -selected kernels	1/15	50	3.3
Hand-rejected kernels	10/16	1, 28, 42, 78, 86, 117, 133, 179, 208, 217	67.7
Hand-selected kernels (finished product)	0/26		0

^a ESM, Electronic sorting machine.

of the 16 samples of hand-rejected kernels (oil stock) were contaminated. Furthermore, nine of the 10 positive samples of manually rejected kernels contained aflatoxin in excess of the guideline level of 20 ppb.

The presence of measurable amounts of aflatoxin in only two of the 16 samples (12%) of the unsorted kernels indicates an incidence of aflatoxin that is in fair agreement with that found in the unsorted, in-shell nuts from the growers (14%). In fact, by statistical analysis similar to that used for in-shell nuts, assuming 400 kernels/lb, the average proportion (p) of contaminated kernels in the unsorted kernels is estimated to be roughly the same as that in the incoming in-shell nuts (2.23×10^{-5} versus 3.78×10^{-5}).

Hand sorting appears to be more effective than electronic sorting in removing aflatoxin-contaminated kernels. Not only was the number of contaminated samples highest in the hand-sorted rejects, but the average aflatoxin concentration was also highest in these rejects. The difference in aflatoxin incidence in hand-sorted rejects and electronically sorted rejects could result from the fact that the kernels rejected by electronic sorting contain a high proportion of broken and sheller-damaged kernels, which dilute the aflatoxin-contaminated kernels that are associated with the "seriously damaged" (moldy and insect-damaged) kernels. Too few samples were analyzed to prove statistically that electronic sorting tends to remove aflatoxin-contaminated nuts.

Since there is a tolerance for seriously damaged kernels in the United States standards for grades of shelled almonds, some samples of finished product eventually may be contaminated with aflatoxin. The higher the tolerance

for seriously damaged kernels, the more likely this will occur. There is little chance of the most seriously damaged kernels being in the finished product, but the present study did not attempt to relate type or degree of defect with aflatoxin. On the basis of the data obtained, it appears that one does not have a very good chance of finding a positive (contaminated) sample in sorted whole nutmeats unless one uses a sample much larger than 15 lb (6.8 kg). For example, if the sorted nutmeats contain 1% of the seriously damaged kernels that are equivalent to those rejected by hand sorting, the probability of finding one or more contaminated samples in the 26 samples of sorted nutmeats used in this study is only 0.225. That is, on the basis of the data (i.e., $p = 1.63 \times 10^{-4}$ for hand-rejected kernels; 1% tolerance for seriously damaged kernels) it can be estimated that 77.5% of the time one can examine 26 samples of 15 lb (6.8 kg) each without finding any contamination.

Processed (finished product) samples. The survey of processed nutmeats was made to determine the frequency and amount of aflatoxin contamination in various finished products available to the consumer. The products sampled for this part of the study were from the following basic categories: whole almonds, sliced almonds, and diced almonds. Aflatoxin occurs to a much greater extent in diced almonds than in sliced or whole almonds (Table 3). Thus, whereas none of the 26 samples of whole nutmeats and only one of the 25 samples of sliced nutmeats were contaminated, 13 of the 27 samples (48%) of the diced nutmeats contained aflatoxin. However, only four of these 13 contaminated, diced samples were above the existing FDA guideline of 20 ppb. Although blanching may tend to lower aflatoxin concentration, this factor was not considered in the analysis of the data because of the small number of samples involved.

A higher tolerance for seriously damaged

TABLE 3. *Incidence of aflatoxin in finished products (processed almonds)*

Product type	Proportion of positive samples (x/n)	Aflatoxin concn	
		Range (ppb)	Avg (ppb)
Whole nutmeats, select, natural	0/26		
Sliced nutmeats, natural and blanched	1/25	0-4	0.2
Diced nutmeats, natural and blanched	13/27	0-119	12.7

kernels used for dicing than for any USDA grade of whole or broken almonds undoubtedly contributes to this finding. To exemplify the situation, a statistical estimate can be made of the proportion of contaminated kernels (whole) in the diced nuts on the basis of the proportion of contaminated samples that were found. Thus, if it is assumed that all diced nut samples were contaminated at the same level and that there were 400 kernels/lb in the raw material from which they were prepared, it can be estimated that the average proportion of contaminated kernels in the diced nuts is 1.09×10^{-4} with 95% confidence limits of 5.6×10^{-5} and 1.9×10^{-4} . That is, on the average there was one contaminated kernel in every 9,200 kernels used for dicing, and the limits for 95% confidence were a lower limit of 1 nut/17,900 nuts and an upper limit of 1 nut/5,300 nuts. Since no samples of sorted, whole kernels were contaminated, it is not possible to make a similar statistical estimate for whole almonds. However, a statistical estimate of the upper limit for a 95% confidence interval of the average proportion of contaminated kernels can be made for whole almonds, and it is 1.9×10^{-5} or no more than 1 nut/52,600 nuts. Since the size of the sample required to include a contaminated kernel is inversely related to the proportion of kernels contaminated, it is evident that much larger samples must be used to survey aflatoxin in whole nutmeats than in diced nutmeats.

Distribution study. The most important factor influencing incidence and sample size requirements is distribution (i.e., degree of non-uniformity) of the contaminant in the product. Aflatoxin distribution undoubtedly varies among the different product categories. Thus, distribution is likely to improve as particle size of the product is reduced, so that aflatoxin should be distributed more uniformly in diced nutmeats than in whole nutmeats. Therefore, in several cases the unground materials remaining from the above surveys of aflatoxin were subsampled to examine aflatoxin distribution in whole and diced nutmeats.

The size requirements for representative sampling can be estimated on the basis of such distribution studies; however, it was not possible to make a reliable estimate from this study, because it was limited by the amount and nature of the material remaining from the surveys. Nevertheless, this cursory study did demonstrate a vast difference between aflatoxin distribution in whole nutmeats and that in diced nutmeats. For example, the whole nutmeats remaining from two in-process samples found to contain aflatoxin (28 and 47 ppb) were

subsampled for distribution analysis. In each case none of the five subsamples (50 g each) contained aflatoxin. The remaining unground, diced nutmeats of three positive samples (5, 75, and 119 ppb) were similarly analyzed, with three, five, and seven subsamples, respectively. In contrast to the results with whole nutmeats, all subsamples of the diced nutmeats contained aflatoxin, although great variation in amount did exist (Table 4). Aflatoxin varied from a trace to a level higher than that found in the 15-lb (6.8-kg) analytical sample. Furthermore, the relative amounts of the four aflatoxins (B_1 , B_2 , G_1 , G_2) differed among the subsamples. Nevertheless, aflatoxin was distributed more uniformly in diced nutmeats than in whole nutmeats.

On the basis of the distribution study, it is evident that the likelihood of finding a contaminated sample in a given lot is much more dependent on aflatoxin distribution than on the proportion of contaminated kernels, which is likely related to the tolerance for damaged nuts. In the survey, the higher proportion of contaminated samples found with diced nutmeats than with whole nutmeats was due primarily to more uniform distribution of aflatoxin in the small nut pieces and only secondarily to the presence of a higher proportion of contaminated kernels (i.e., from the higher tolerance for damaged nuts). Because of the difference between aflatoxin distribution in diced nutmeats and in whole nutmeats, much smaller samples of diced nutmeats than whole nutmeats should be re-

TABLE 4. *Distribution study of diced almonds*

Description	Wt	Aflatoxin (ppb)				
		B_1	B_2	G_1	G_2	Total
Small-diced, natural	15 lb	88	19	10	2.0	119
	50 g	1.2	0.4	0	0	1.6
	50 g	42	4.4	25	2.6	74
	50 g	41	12	0	0	53
	50 g	2	0.4	1.0	\pm^a	3.4
	50 g	19	6.5	0	0	25.5
	50 g	4	1	0	0	5
	50 g	153	33	0	0	86
	15 lb	62.5	10	2.5	0.4	75.4
Small-diced, natural	50 g	1.2	0.4	\pm	0	1.6
	50 g	7.3	2.1	0	0	9.4
	50 g	65	20	0	0	85.0
	50 g	33	9.0	0	0	42.0
	50 g	125	40	5.0	\pm	170
	15 lb	4	1	\pm	0	5
Fine-diced, blanched	50 g	0.5	\pm	\pm	0	0.5
	50 g	\pm	0	\pm	0	\pm
	50 g	15	5	0	0	20
	50 g	15	5	0	0	20

^a \pm , Trace.

quired to monitor aflatoxin. Whereas 15 lb (6.8 kg) may be an adequate size for monitoring aflatoxin in diced nutmeats, it is estimated that a sample of 10 to 100 times this size would be necessary for equal assurance of properly evaluating a lot of whole nutmeats. Further study is necessary before any precise estimate can be made of sampling requirements for monitoring aflatoxin in almonds.

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